RECENT EXPERIMENTS ON REGENERATION.

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DURING the summer of 1896 at the Marine Biological Laboratory the writer, while engaged upon the life history of some of the Hydrozoa, took occasion to repeat certain of the experiments of Loeb,[1] Bickford,[2] and others upon regeneration and heteromorphosis among hydroids, and to extend them to several other forms. In the following paper it is proposed to submit a synopsis of the several experiments made, with such results as have sufficient definiteness to justify record.

The work upon hydroids was restricted for the most part to the genera Eudendrium, Pennaria, and Clava ; though some of the experiments of the investigators named upon Tubularia, Margelis, etc., were repeated, and with results quite as they had reported. The work upon Eudendrium was quite as demonstrative as either of the former, and that notwithstanding the fact of its indisposition to take kindly to the artificial conditions of the aquarium and its rather complex character. Not only does Eudendrium readily regenerate excised parts, such as stalk, hydranth, root, etc., but it exhibits equally marked heteromorphism. Experiments upon the other genera mentioned were of the same general nature and results. Those upon Clava were somewhat limited, owing to the very limited supply obtainable during the present summer. No particular attention was devoted toward ascertaining immediate causes by which to account for the phenomena. That external conditions have much to do with certain aspects of it I have no doubt but that they are the chief, or primary, conditions seems at least an open question. Specimens placed under identically similar conditions do not respond with equal promptness nor with similar results. Many facts would seem to indicate the operation of intrinsic factors. But of this it is

[1] Biological Lectures, 1893, p. 37.

[2] *Journ. of Morph.*, vol. ix, p. 417.

not the purpose here to inquire in detail. Further investigations, some of which are under way, will be necessary to afford sufficient data for any adequate judgment.

The particular class of experiments to which I purpose to direct attention at this time pertains to another group of organisms, and one upon which, so far as I am aware, nothing directly has been done, though incidentally it has been referred to in connection with another subject, and will be duly noted in another part of the paper. The organisms referred to are the medusae. In connection with the previous work already noted, the thought of extending these experiments to the medusae occurred more than once, but owing to their peculiar delicacy and highly specialized character, was dismissed as of doubtful practicability. The presence, however, of considerable numbers of Gonionemus vertens, a preliminary report upon which was made during the previous year by Dr. Murbach, and the capacity of which to endure confinement in small aquaria was rather marked, revived the previous conception, and after reflection it was determined upon with some hesitation.

Accordingly a small number of these medusae were obtained and placed in small table aquaria, and upon them a series of extremely simple experiments made, such as the excision of a few tentacles, notching the margins, etc., more from a spirit of curiosity than of expectation. When, therefore, upon the following day I noted that the mutilations were healed in several cases and that there was an evident tendency toward a restoration of excised parts, interest was only exceeded by surprise. However, I found, upon a brief review of the work of Romanes[1] on the *Nervous System of Jellyfish*, etc., that he had called attention to the fact of the capacity of these organisms to regenerate mutilated tissues, but no work was done in connection with the observation. Later Eimer also made certain experiments upon jellyfishes of a similar sort, but without recording any tendency toward active physical regeneration, though demonstrating the recovery of nervous activity.[2] I may incidentally note the fact that in none of my

[1] Jellyfish, Starfish, and Sea Urchins, p. 103.

[2] Organic Evolution, pp. 345 *et seq.*

experiments did I observe that apparent paralysis resulting from the excision of the marginal nervous system which he found so striking. However, the fact that his work was done chiefly upon Scyphomedusae, while Gonionemus apparently belongs to the Hydromedusae, may sufficiently account for the difference. While in my experiments there seemed to be a sort of shock induced by extensive mutilations, it was, however, in no case of any considerable duration or effect upon the specimens.

With these simple results as an index of possibilities a considerable number of medusae were secured and a series of systematic excisions and mutilations was begun. The following synopsis will serve to indicate the scope and character of the experiments, though in most respects the record must be regarded as of the nature of a preliminary report, since the histological investigations to determine the deeper results upon the tissues are not yet sufficiently complete to be presented, and, moreover, some of the primary experiments need verification under other conditions than were then possible, or than the time at my disposal after they were undertaken was sufficient to complete. In view of the interest and significance of the work in its relations to current discussions, it has seemed advisable to give publicity to the facts thus far established, reserving to a later time further details upon the subject which may come from more extensive and critical experimentation.

Gonionemus vertens is a medusa of very interesting habits, and of a form and size well adapted to the work undertaken. It may not be amiss in this connection to record the fact that, so far as published records of its distribution are known, it had not, prior to the summer of 1895, been known on the Atlantic coast. I am informed by Prof. C. C. Nutting that during the summer of 1896 he found evidence at Newport of its occurrence there, but at what time I could not learn.

In Fig. 1 is shown in a somewhat diagrammatic form the more characteristic features of the medusa. In size it varies considerably, owing probably more to age than any other cause, averaging from one to two centimeters in marginal diameter.

Not pausing to notice the simpler experiments already referred to, such as the slight incisions, or notching of the margins, excision of tentacles, etc., the first experiments consisted in excising portions of the margin of the umbrella, as indicated in Fig. 2. These were repeated upon about twenty specimens, removing portions of the body between the radial canals and portions including them. The regeneration of such portions was usually quite prompt, varying from two to four days, depending chiefly and naturally upon the size of the portion to be restored. In every case the regeneration seemed to be perfect, including radial canals, velum, and tentacles.

In a few specimens all the tentacles were removed by plucking them singly from their very roots with fine forceps. In

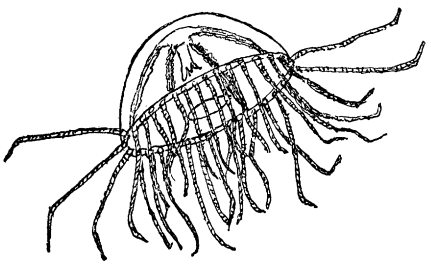

FIG. 1.

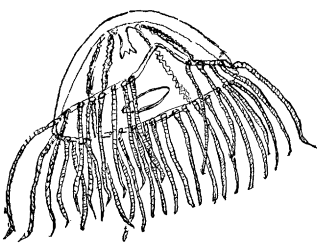

FIG. 2.

these cases regeneration followed in about the same time as in the others, but the tentacles were more slender and delicate than the ordinary ones.

In the next series the manubrium was excised close up to the stomach cavity, and indeed in some cases including the entire thickness of the aboral body wall. The results were as before, the manubrium being regenerated with promptness and completeness and in about the same time, depending as before upon the amount of matter to be restored. It may be noted in this connection that the excised manubria themselves continued to live, even for days, and to move about by a slow, creeping sort of motion, but did not show any appreciable tendency toward regeneration. The same was true of small particles from almost any part of the body, such as tentacles, bits of margins, etc. No special attention was given to determining from how small a portion of the animals regeneration

might be secured, but from the general tenor of the experiments I should doubt whether anything less than a full quarter of the body would reproduce a new individual. This, however, is merely inference based on the general facts observed. Specific experiments to be undertaken later will possibly show very different results.

In the third series the experiments consisted in vertical sections through the median portion of the body, dividing it as nearly as possible into halves, as indicated in Fig. 3, and with the result that each half became an independent and perfect medusa. In this case the restoration was somewhat peculiar. It would seem to be a sort of *recovery* of form and function rather than regeneration in the usual sense of that term. After the first brief shock of the operation, which in many cases was scarcely noticeable, was overcome, there was an evident effort of the half-medusa to assume the normal form by a contraction of the body so as to bring the cut edges of the bell together, the approximations of the edges taking place from above downward, or peripherally, and with the subsequent union or healing of the approximated margins. Complete restoration, resulting in the assumption of the original form, occurred in from three to five days. The new medusae were in most respects quite similar in form and action to the original, though of course of only about half the size. The time at my disposal was insufficient to observe whether there was subsequent growth of the specimens. In the recovery of the specimens I was not able, moreover, to observe any disposition to regenerate the additional radial canals necessary to complete the symmetry of the original. This, however, does not seem to be an important matter, since there does not seem to be a special necessity for a definite number. And in this connection it may be worth while to note a very considerable variation in normal forms as to the number of radial canals.

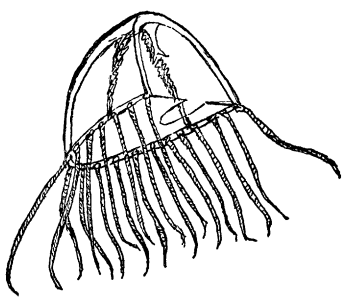

FIG. 3.

In Gonionemus, as in most Hydromedusae, the number is four. But in the specimens studied in this connection it was not rare to find three or five or six. This is the more remarkable in that Bateson[1] has been able to record but rare instances of such variation among Hydromedusae, instancing those noted as among the most striking illustrations of the "discontinuity of meristic variation."

A fourth series consisted in sections of the medusa in a horizontal plane, as indicated in Fig. 4. As will be perceived, this is clearly the most crucial of the entire series, since the animal is divided into such parts that if regeneration occur at

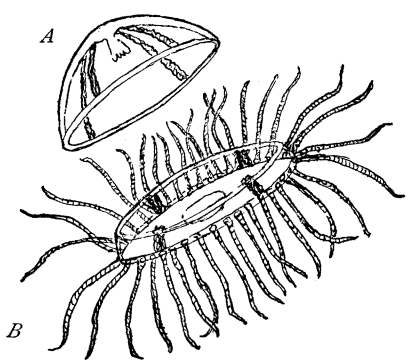

FIG. 4.

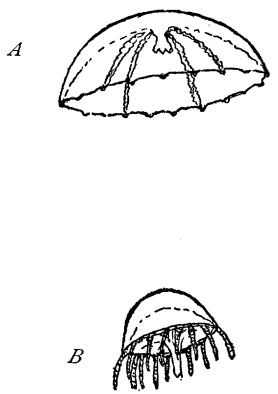

FIG. 5.

all it must be *de novo*, from tissues devoid of any direct identity of form or function. But crucial as is the test, it was none the less successful. In Fig. 4, *A* and *B* represent the central and marginal portions of the divided medusa. In Fig. 5, *A* and *B* represent the corresponding portions after regeneration had occurred. *A* in this figure needs no special explanation. That which is first regenerated in this portion is the marginal canal. Next following this is the regeneration of the tentacles. As will be noticed from the figure, they are but rudimentary or bud-like. The unprecedented hot weather which occurred just at this time made extremely difficult the preservation of specimens in a healthy condition under the artificial conditions of

[1] Bateson: Material for Study of Variation, pp. 424 *et seq.*

the aquarium, and many perished before regeneration of these organs had gone forward to any considerable extent. Of its reality, however, there is not the least doubt. The rate of regeneration in these cases was much slower than in the former, as would be naturally expected, in most cases requiring a fortnight to afford conclusive indications of the new organs.

Most marked in many respects is the condition indicated in *B* of Fig. 5, the resultant of *B*, Fig. 4. That is, the excised margin had become a new medusa. But in this case, as in the vertical sections, the process appeared as more a restoration of form through a marked and continued contraction of the marginal walls, and a final union of their upper margins to form the dome of the new medusa. Again there seems to have been no evidence of growth in substance, which would in this case have been impossible, since the absence of mouth or gastric cavity would render the taking of food entirely out of question, and there would also be a draft upon any reserve energy in the tissues in the mere maintenance of life and the usual waste incident to existence. And this fact of itself makes the results more interesting; namely, that in a minute fragment of the nature of the one under consideration there seems to be an intrinsic potency to recast itself into the morphological equivalent of the original; and that this disposition was manifest in even the minutest portion from any part of the body. So marked, indeed, was this tendency that it almost seemed as if there were present some occult organic crystallization prepotency, if such a phrase or comparison be tolerable.

Such in brief is an outline of the facts concerned in the experiments. Their significance in relation to others of like character and to problems of current biological importance will be more or less apparent without special emphasis. It may not be amiss, however, to refer briefly to some theoretical considerations upon which they would seem to have special bearing.

Concerning the problem of the more primitive character of hydroid or medusa no additional light is afforded. If the more highly developed and specialized nature of the medusa is

appealed to as evidence of its derivation from the hydroid type, the capacity of the medusa for regeneration and the tenacity with which it maintains the medusan features would seem to point with equal force in the opposite direction. While this alone is not sufficient to settle a problem involving so many factors, it none the less suggests the propriety of caution and the necessity for further evidence than has yet been adduced before any dogmatic or final statement is made.

Concerning heteromorphosis the experiments were entirely negative. A definite polarity seemed evident in every part of the organism. It mattered not how small the fragment, or from what source, or however varied the conditions under which placed, the results uniformly indicated a definite intrinsic orientation.

If this might seem to imply a more fixed and constant heredity and therefore to point toward a more primitive condition than is usual in the hydroid, it is sufficient to call attention to the no less fixed and inflexible polarity of Hydra. It would seem, therefore, that on these points the facts are to be taken simply for what they are, and not as affording any definite basis upon which to speculate.

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